

CLAIMS

I claim:

1. A hybrid gene cDNA library comprising a series of vectors, each vector comprising a DNA molecule having at least one selectable marker sequence and a sequence encoding a hybrid protein region, wherein the hybrid protein region comprises,
 - a) a regulatable DNA sequence,
 - b) a multiple cloning site immediately 3' to the regulatable DNA sequence, wherein the multiple cloning site does not encode a translational termination sequence, and
 - c) a DNA sequence encoding at least one common peptide placed 3' to the multiple cloning site, wherein the common peptide encoding sequence does not contain a translation initiation codon, and wherein the each vector of the library additionally comprises a single cDNA molecule inserted at the multiple cloning site wherein each of said cDNA molecules is obtained from a cDNA population generated using random primers.

- 2 The hybrid gene cDNA library of claim 1 wherein each vector additionally comprises one or more origins of replication active in bacteria cells.

3. The hybrid gene cDNA library of claim 1, wherein each vector additionally comprises one or more origins of replication active in yeast cells.
4. The hybrid gene cDNA library of claim 1, wherein the hybrid protein region additionally comprises a sequence which encodes a transcriptional termination sequence placed immediately 3' to the DNA sequence encoding the at least one common peptide.
5. The hybrid gene cDNA library of claim 1, wherein the regulatable DNA sequence is the rat Glucocorticoid Response Element
6. The hybrid gene cDNA library of claim 1, wherein the regulatable DNA sequence is an Estrogen Response Element.
7. The hybrid gene cDNA library of claim 1, wherein the common peptide is encoded by a DNA molecule comprising sequences encoding all or portions of the GAL4 yeast transcriptional activator and six successive histidine residues.
8. The hybrid gene cDNA library of claim 1, wherein the common peptide is encoded by a DNA molecule comprising sequences encoding all or portions of the

GAL4 yeast transcriptional activator and a nuclear localization sequence from the SV40 virus.

9. The hybrid gene cDNA library of claim 1, wherein the common peptide is encoded by a DNA molecule comprising sequences encoding an immunological epitope from adenoviral hemagglutinin.

10. The hybrid gene cDNA library of claim 1, wherein each of the vectors additionally comprises one or more origins of replication active in yeast cells and one or more origins of replication active in bacterial cells, wherein at least one yeast origin of replication is derived from the natural 2-micron yeast plasmid.

11. The hybrid gene cDNA library of claim 1, wherein the selectable marker sequences are selected from the group consisting of the bacterial ampicillin resistance gene and the yeast TRP 1 nutritional auxotrophy gene.

12. The hybrid gene cDNA library of claim 1, wherein the selectable marker sequences are selected from the group consisting of the bacterial kanamycin resistance gene and the yeast URA3 nutritional auxotrophy gene.

10071135-020602

13. The hybrid gene cDNA library of claim 4, wherein the transcriptional termination sequence is derived from the yeast ADH 1 gene.

14. A method of producing hybrid proteins from a hybrid gene cDNA library comprising:

a) providing a purified sample of a vector comprising a DNA molecule having at least one selectable marker sequence and a sequence encoding a hybrid protein region, wherein the hybrid protein region comprises,

i) a regulatable DNA sequence,

ii) a multiple cloning site immediately 3' to the regulatable DNA sequence, wherein the multiple cloning site does not encode a translational termination sequence, and

iii) a DNA sequence encoding at least one common peptide placed 3' to the multiple cloning site, wherein the common peptide encoding sequence does not contain a translation initiation codon,

b) isolating a mRNA template population of interest;

c) synthesizing a cDNA population from the mRNA template population using random sequence oligonucleotide primers;

d) adding cloning linkers to the cDNA population,

e) cleaving the vectors at the multiple cloning site,

f) inserting the cDNA population molecules into the cleaved vectors, to create a hybrid gene cDNA library,

g) transforming bacterial cells with the hybrid gene cDNA library and selecting transformed cells,

h) purifying the hybrid gene cDNA library from the transformed bacterial cells;

i) transforming yeast cells with the hybrid gene cDNA library and selecting transformed cells, and

j) allowing transformed yeast cells to produce a hybrid protein.

15. The method of claim 14, wherein the bacterial cells transformed with the hybrid gene cDNA library are *E. coli* cells.

16. The method of claim 14, wherein the vector encodes a common peptide sequence comprising six successive histidine residues and the hybrid protein is purified from the yeast cells using affinity purification.

17. A method of performing a yeast two-hybrid assay comprising:

a) providing a hybrid gene cDNA library comprising a series of vectors, each vector comprising a DNA molecule having at least one selectable marker sequence and

a sequence encoding a hybrid protein region, wherein the hybrid protein region comprises,

i) a regulatable DNA sequence,

ii) a multiple cloning site immediately 3' to the regulatable DNA sequence, wherein the multiple cloning site does not encode a translational termination sequence, and

iii) a DNA sequence encoding at least one common peptide placed 3' to the multiple cloning site, wherein the common peptide encoding sequence does not contain a translation initiation codon, and wherein the each vector of the library additionally comprises a single cDNA molecule inserted at the multiple cloning site wherein each of said cDNA molecules is obtained from a cDNA population generated using random primers,

wherein the common peptide comprises a DNA activation domain,

b) providing yeast cells comprising a second hybrid protein comprising a DNA binding polypeptide and a bait polypeptide and additionally comprising a DNA molecule comprising a sequence to which the DNA binding polypeptide may bind, a sequence activatable by the DNA activation domain and a reporter sequence,

c) transforming the yeast cells with the hybrid gene cDNA library and selecting transformed yeast cells, and

d) performing an assay to detect activation of the reporter sequence.

1007136.020602

18. The method of claim 17 wherein the DNA activation domain is derived from the yeast the GAL 4 activation domain, and the reporter sequence is derived from the yeast GAL 4 gene.

19. The method of claim 17, wherein the vector comprises a TRP 1 nutritional auxotrophy gene as the selectable marker sequence and the yeast cells are trp 1 mutant yeast cells.

20. The method of claim 17, wherein the vector comprises a URA 3 nutritional auxotrophy gene as the selectable marker sequence and the yeast cells are ura 3 mutant yeast cells.

21. The method of claim 17, wherein the common peptide additionally comprises a nuclear localization sequence.

22. The method of claim 21, wherein the nuclear localization sequence is a nuclear localization sequence from the SV40 virus.

2007-11-20 14:00:00